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CROWN-GALL OF APPLE AND PEACH WITH NOTES ON THE BIOLOGY OF BACTERIUM TUMEFACIENS

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CROWN-GALL OF APPLE AND PEACH WITH NOTES ON THE BIOLOGY OF BACTERIUM TUMEFACIENS

DONALD REDDICK AND V. B. STEWART

THE CROWN-GALL DISEASE

The crown-gall disease, caused by *Bacterium tumefaciens* Smith & Townsend,[1] occurs on a great variety of plants and over a wide geographical range. It has been noted particularly in fruit culture from practically every fruit district in the United States, and the very general opinion expressed in the literature on this subject is that the disease is a dangerous one and its introduction into a planting is greatly to be feared.

The evidence bearing on the economic importance of the disease is largely observational and few experimental data are at hand to show its exact importance. The observational data are fully summarized by Smith, Brown, and Townsend[2] and need not be repeated here. In general, it may be concluded from these authors' digest of the literature that apples are not seriously affected but that peaches and many other plants are. These writers are inclined, however, to include apples (page 197 of the bulletin cited) in the list of plants that should be inspected for crown-gall, and to recommend rejection of trees showing galls or hairy root. They suggest that even if apple trees are not materially affected by crown-gall, the distribution of trees bearing galls will serve to spread the disease to new localities and to other hosts which are more susceptible and which may be injured thereby. Neither point seems to be well taken when one considers (1) the very wide distribution of the disease at the present time, and (2) that the apple constitutes a relatively permanent crop which is not interplanted except for a few years and then preferably with cultivated crops such as potatoes, beans, corn, and the like, that is, plants which do not develop the disease under natural conditions.

In a more recent publication, Erwin F. Smith[3] states that "when death results it is not due to the direct action of the bacteria, but to

[1] Attention is called to the fact that this organism is named *Bacterium tumefaciens* in conformity with the nomenclature of Erwin Smith, which is in common use among plant pathologists. According to the nomenclature commonly followed by bacteriologists, however (see *Bergey's Manual of Determinative Bacteriology*, 1923), this organism would be called *Phytomonas tumefaciens*.

[2] Smith, Erwin F., Brown, Nellie A., and Townsend, C. O. Crown-gall of plants: its cause and remedy. U. S. Dept. Agr., Bur. Plant Ind. Bul. 213:1–215. 1911.

[3] Smith, Erwin F. Further evidence that crown gall of plants is cancer. Science 43:871–889. 1916. (The quotation cited is on page 876.)

other factors, *e.g.*, nutritional defects, and secondary parasitisms," and it is to be expected, therefore, that climatic conditions, soil conditions, and various other factors may enter to cause even greater variations in different regions than would result if only the one complication were involved. For this reason, regional tests must be made to determine the destructiveness of the disease for any particular set of conditions.

In this connection it is to be recalled that some years ago F. C. Stewart [4] stated before a convention of fruit growers that "in New York, at least, apple crown gall is an unimportant disease." Stewart cites the C. H. Stuart orchard at Newark, New York, as an example. It has been objected that C. H. Stuart was engaged in the nursery business at the time when the orchard was set (the inference being that he was therefore not disinterested), and that apparently no healthy trees were set for comparison. The former fact practically assures that the trees really had crown-gall disease, and the latter is a natural mistake sometimes made by others than merely practical men.

The controversy concerning the number of trees originally set in the Stuart orchard, and particularly the question as to whether one hundred of the original trees died, possibly from crown-gall, led one of the writers (R) to visit the orchard. The following facts were either ascertained from E. V. Pierson (the present owner and a son-in-law of C. H. Stuart), or determined by personal observation. The orchard is one and a half miles directly north of Newark on Main Street. It is on the right-hand side and a little back from the road, and is permanently marked by a large stone some three feet in diameter which lies at the southwest corner. The exact limitations of the original planting of galled trees cannot be determined. There are more than a thousand trees in the orchard, all apparently of the same age and of the same general appearance. The block, therefore, constitutes a satisfactory experiment, because at least as many gall-free trees were used as trees bearing galls. The number of galled trees planted originally is of little consequence, but Mr. Stuart's statement to F. C. Stewart may be accepted as the more likely number, namely, 500 trees. The galled trees in particular constitute a mixed lot of varieties. Baldwin, Greening, Sutton, and Ben Davis were recognized, and others are present. The remainder of the orchard seems to be somewhat mixed, also. The experiment indicates that, to date, crown-gall has done no material damage. Allowing for the numerous varieties set indiscriminately, the orchard is pronounced "average" by W. H. Chandler, Professor of Pomology at Cornell University. Trees have died here and there and have been replaced. Excepting for a wet area which scarcely could have been included in the block of galled trees, the number of missing trees is not greater in one area than in another.

[4] Stewart, F. C. Interesting facts about crown gall of apple and peach. West. New York Hort. Soc. Proc. 53:97–98. 1908.

Recently, experimental data have been published on crown-gall experiments with apples, from the States of Iowa[5] and Montana.[6] The experiments were planned and executed on a large scale. In Montana it appears that while there was no noticeable effect on the vigor of the trees, nevertheless the root development of galled trees was not good, and it is thought that this would have become apparent when the trees began to bear heavy crops. In Iowa numerous growth data were recorded, and these show without exception that galled trees did not grow so fast as did trees free from gall. It should be noted, in this connection, that the method employed was one which has not yet received general indorsement by horticulturists.

An objection to all the experiments reported, including the one about to be recorded, is that the real test comes only with the production of fruit (because the trees are grown for the fruit they bear, not for the wood they produce). A well-planned experiment to determine the effect of crown-gall should extend well into or through the life of the plant. This would be a very long time for apple trees, but could be done relatively easily with peaches.

A proper interpretation of any of the crown-gall experiments thus far reported is rendered difficult by the fact that the experimental trees used have consisted of a miscellaneous collection for which there are no data as to original source, particularly as to the exact character and origin of the stock on which the trees were grown. The Iowa experiments illustrate this very well. One of the conclusions of Greene and Melhus is that Wealthy is more susceptible to crown-gall than is Jonathan. It is difficult to understand how this can be, since the varieties were grafted on seedling stock and an examination of the illustrations indicates that the galls are confined almost entirely to the stocks. It thus becomes necessary to assume an influence of scion upon stock which is not yet admitted by horticulturists. Under the circumstances it may be assumed with equal probability that the conditions of the experiment were such that a large variable factor was not brought under control. The Iowa experiment is further complicated by the fact that the plot of land in which the trees were planted had grown nursery stock some of which was affected with crown-gall, and that the plot is so located that it, or at least a part of it, received the wash from adjacent affected trees. Furthermore, the report does not state whether the trees were planted in such a way as to eliminate variations in soil and in growth conditions. It must be remembered in this connection that in average orchard planting the chance of infection after the trees are permanently set is very small.

[5] Greene, Laurenz, and Melhus, I. E. The effect of crown gall upon a young apple orchard. Iowa Agr. Exp. Sta. Research bul. 50:145–176. 1919.

[6] Swingle, D. B., and Morris, H. E. Crown-gall injury in the orchard. Montana Agr. Exp. Sta. Bul. 121:123–139. 1918.

The crown-gall disease seems to have been much more prevalent in New York some fifteen or twenty years ago than it is at the present time. There was much more agitation about the disease at that time, but this may be accounted for in part by the fact that inspection service for San José scale was being instituted and enforced, and that this inspection involved also examination for and rejection of trees having crown-gall. Aside from this, however, one of the writers (S), who was interested in diseases of nursery trees, recalls that in 1909 the fields in one large nursery from which apple trees had been dug were thickly strewn with trees that had been rejected at digging time because of crown-gall.[7] It was this condition, in part, that led to the experiments herein reported. But in 1910, when trees were sought for the experiments, very few were to be found and there was some difficulty in procuring the necessary stock. This condition has prevailed in subsequent years. One factor in nursery management may have contributed to this desirable condition, namely, that there has been an increasing tendency on the part of the nurserymen to grow trees in rotation with farm crops, thus allowing for the death of *Bacterium tumefaciens* in the soil or on plant parts. Another factor which may be involved is that most apple trees in New York are budded on French stock. One of the writers (S)[8] has expressed the opinion that French-grown stock is less susceptible to crown-gall than is American-grown stock, and it seems obvious that the practice of budding in the field, which is the common practice in New York, gives less opportunity for widespread infection than does that of bench grafting.

PLAN OF THE CROWN-GALL EXPERIMENT

In order to determine, if possible, the importance of crown-gall on apple and peach trees under the conditions existing at Ithaca, New York, an experiment was planned, the details of which were as follows:

The land for the experiment lay in a low field which had been in grass for a number of years previously. There are hills on all sides, but there is suitable drainage so that the soil is never too wet for good growth. The soil is a good loam, running out into stony loam on the side where the rows are marked *A* in figure 1. The soil is suitable for the growth of both apples and peaches, but the location is unsuited for peaches. Peaches are grown successfully a few miles from Ithaca, but there is little chance of securing crops in the immediate vicinity, chiefly because of low temperature in the winter. This was not fully realized at the beginning of the experiment.

The land was ploughed and cultivated each year and a sown cover crop usually was employed.

[7] It is likely, though, that some of these trees bore galls produced by an aphis (*Schizoneura lanigera*), and this is the opinion expressed by one large nurseryman.

[8] Stewart, V. B. Exclusion legislation and fruit tree production. Phytopath. 8:360–364. 1918. (The citation refers to page 362.)

THE EXPERIMENT WITH APPLES

Apple trees, of several varieties, bearing evident lesions of crown-gall were procured in the autumn of 1910. On April 20, 1911, photographs were made of the trees[9] and the trees were set in the garden. On May 6, healthy trees were procured, photographs were made, and the trees were interspersed with the diseased trees as shown in the accompanying plan (figure 1). All the trees were two years from the bud and were on imported French stock. Practically all of them, except for the galls, would

	1	2	3	4	5	6	7	8	9
A	**S**	**We**	S	**We**	**S**	**We**	**S**	**We**	**S**
B	**We**	**TO**	**We**	TO	**We**	**TO**	Wa	**TO**	**Wa**
C	**B**	**Wa**	**B**	**Wa**	B	**A**	**B**	**Wa**	**K**
D	**Wa**	**WR**	**Wa**	**WR**	**Wa**	**K**	WR	**K**	K

FIGURE 1. PLAN OF THE APPLE CROWN-GALL EXPERIMENT

Each character represents a tree and indicates the name of the variety according to the following key: *A*, Alexander; *B*, Baldwin; *K*, Tompkins King; *S*, Northern Spy; *TO*, Twenty Ounce; *Wa*, Wagner; *We*, Wealthy; *WR*, Wolf River. Bold-face letters represent galled trees; light-face letters represent healthy trees

have been graded as best-quality trees. The notable exception is tree C-6, variety Alexander, which was small in diameter and bore a very large gall at the crown.

The trees were pruned very lightly from year to year, and in all cases the pruning consisted in the entire removal of undesirable limbs or branches. No heading-back was done.

A record of the general appearance of the trees was made twice each year, always without notes of the previous condition. For the most part the records are normal and may be tabulated without comment. One tree, C-3, was girdled by mice in the winter of 1913-14. A renewal developed but was winterkilled the following winter. The tree was removed on May 28, 1915, when it was found that the galls had enlarged appreciably (figure 2). The record of this tree previous to injury is shown in table 1.

The records for the apple trees are given in table 1. Before examining the records or deriving any conclusion, the reader should have in mind a number of items. (1) It is now evident that the experiment was not planned on a large enough scale to give the best results, and particularly there were not enough healthy trees planted for comparison. This was not an oversight on the part of those planning the experiment, but rather

[9] The photographs are on file in the Department of Plant Pathology at Cornell University under the number 5727. None is reproduced here because the negatives are uniformly poor and suitable reproductions cannot be made. Camera and plates were tested in order that uniformly good photographs might be obtained without waiting to develop the negatives. When all negatives were found to be uniformly bad, it appeared probable that the shutter of the camera had been tampered with in the interim. The original negatives show the location and size of the galls very readily, and they proved most useful when the trees finally were removed.

was due to space limitations. (2) The varieties employed in the test were so numerous and the growth habits so different that comparisons between varieties would lead to error. On the other hand, the number of trees of any one variety was scarcely large enough to allow for satisfactory comparisons. (3) The yield of the trees was expected to give quantitative data. The trees had only just come into bearing, and so these data were not obtainable at the time when, of necessity, the experiment was terminated. It is not likely that the data would have been of sufficient

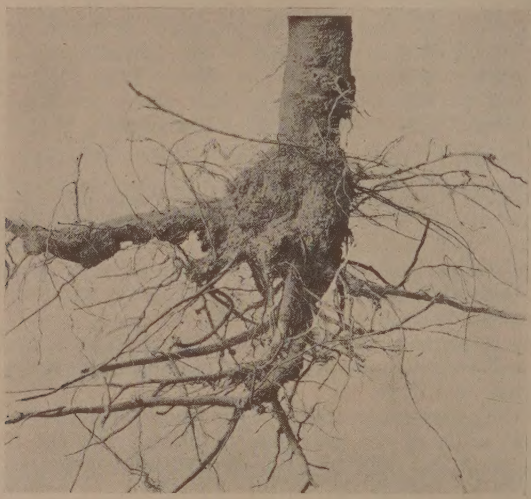


FIGURE 2. BALDWIN TREE C-3

The galls had increased somewhat in size, but there was no indication that they had interfered with the development of the tree

extent to be very reliable. (4) The apple-tree borer (*Saperda candida*) proved a great pest. Sometimes as many as six larvae were found in a single tree. If these larvae are removed, little injury will follow; but if one or two are missed—and this apparently is almost inevitable—great damage may be wrought within a few weeks. Most of the fluctuations in general appearance of trees from year to year, without question, are attributable to this borer. (5) Frost, rabbits, mice, and other fortuitous circumstances reduced the size of some trees so that, with the small numbers involved, it did not seem worth while at the end even to weigh the trunks and the crowns, although this could have been done very easily.

TABLE 1. ANNUAL RECORD OF GROWTH AND DEVELOPMENT OF HEALTHY APPLE TREES AND OF TREES AFFECTED WITH CROWN-GALL

(The trees were set in the spring of 1911 and records were made from 1912 to 1919 inclusive. The final record was made on May 20, 1920. In examining the trees for general condition and appearance, four classes were recognized: *e*, excellent; *g*, good; *f*, fair; *p*, poor. Healthy trees are designated by an asterisk)

Variety	Location	General condition of growth for the respective seasons								Condition of roots at digging time
		1912	1913	1914	1915	1916	1917	1918	1919	
Northern Spy..	A-1	p	p	f	g	f	f	f	e	g. No gall
Northern Spy..	*A-3	f	g	e	e	e	e	e	e	e. No gall
Northern Spy..	A-5	g	g	e	e	e	e	e	e	e. No gall
Northern Spy..	A-7	f	g	e	e	e	e	e	e	f. Hard gall at crown, size of a liter flask
Northern Spy..	A-9	g	g	e	e	e	e	e	e	g. No gall
Wealthy.....	A-2	f	g	g	g	g	g	g	e	g. Small galls on one large root
Wealthy.....	A-4	g	g	e	e	e	e	e	e	g. Two small hard galls at crown; small galls on two roots with hairy root
Wealthy.....	*A-6	f	g	e	e	e	e	e	e	g. No gall
Wealthy.....	A-8	g	g	e	e	e	e	e	e	e. No gall
Wealthy.....	B-1	e	g	g	g	g	g	g	e	e. No gall
Wealthy.....	B-3	e	g	e	g	...[1]	.....	.....	.....	.....
Wealthy.....	B-5	f	f	f	f	f	f	f	f	e. One hard gall (egg) on trunk; smaller galls on several roots
Twenty Ounce..	B-2	e	e	e	e	e	e	e	e	e. No gall
Twenty Ounce..	*B-4	f	g	g	e	e	e	e	e	e. No gall
Twenty Ounce..	B-6	e	g	e	e	e	e	e	e	Broken off at crown
Twenty Ounce..	B-8	g	g	....	e	e	e	e	e	g. No gall
Wagner.....	*B-7	f	g	e	e	e	e	e	e	g. No gall
Wagner.....	B-9	f	g	....	g	e	g	e	e	g. No gall
Wagner.....	C-2	g	g	e	e	e	e	e	e	e. No gall
Wagner.....	C-4	f	g	e	g	g	g	g	e	g. Remnant of hard gall in crown; small galls (egg) on two roots
Wagner.....	C-8	g	g	f	f	g	f	g	f	g. Hard gall (egg) in crown and on one root; small gall on one root
Wagner.....	D-1	g	g	g	e	e	e	e	e	g. No gall
Wagner.....	D-3	e	e	e	e	e	e	e	e	g. No gall
Wagner.....	D-5	g	g	e	e	e	e	e	e	g. One small (metastatic?), recent, hard gall on trunk above ground
Baldwin.....	C-1	g	g	e	e	e	e	e	e	e. No gall
Baldwin.....	C-3	g	g	...[2]	.....	.....	.....	.....	.....	.....
Baldwin.....	*C-5	e	e	e	e	e	e	e	e	g. No gall

[1] Girdled by borer; removed October 11, 1916; gall in crown 8 centimeters in diameter.
[2] Girdled by mice; removed May 28, 1915; gall enlarged. (See figure 2.)

TABLE 1 (*concluded*)

Variety	Location	General condition of growth for the respective seasons								Condition of roots at digging time
		1912	1913	1914	1915	1916	1917	1918	1919	
Baldwin	C-7	g	g	g	g	g	g	g	f	g. No gall
King	C-9	e	g	e	e	e	e	e	e	g. Hard gall (egg) at surface of ground
King	D-6	g	e	e	e	e	e	e	e	g. No gall
King	D-8	e	g	e	e	e	e	e	e	e. No gall
King	*D-9	f	g	e	e	e	e	e	e	g. No gall
Wolf River	D-2	e	e	e	e	e	e	e	e	e. Doubtful galls on a few small roots
Wolf River	D-4	e	e	e	e	e	e	e	e	e. Small gall (egg) in crown
Wolf River	*D-7	e	e	e	e	e	e	e	e	e. No gall; stock enlarged (natural?)
Alexander	C-6	e	e	e	e	e	e	e	e	e. Stock enlarged on one side; hard gall present

The trees were removed on May 24, 1920. About two-thirds of them were pulled by hitching a team directly to them; the remainder were pulled with blocks. There was not the least indication that gall-free trees pulled more easily than those originally bearing galls. One tree, B-6, broke off at the surface of the ground, but this seems to have been due to weakening from borers and to the large size of the roots.

On examining the roots it could not be detected, from their appearance, size, or length, that there was any difference between the roots of galled trees and those of trees free from galls. The most surprising thing revealed by examination of the roots was that in many instances the original galls had disappeared completely. This was true in practically all cases in which the original tree had a long rootstock and the gall was located near the base of the stock. All the main roots issued from the stock near the surface of the ground, due perhaps to the nature of the subsoil. In most cases in which the gall was located on a lateral root, both root and gall had disappeared, but the same disappearance of original roots was noted in healthy trees also.

Summary for the apple experiment

The data in table 1 may be summarized in a few words. In no case is there indication that the presence of crown-gall on the roots of these apple trees interfered in any way with growth. The galls had largely

disappeared, and judging by this test the crown-gall disease on apples seems, in this locality, to be chiefly a matter of concern to the nurseryman.

Even in the nursery it is not certain that the disease is of great consequence for apples. It will be noted that most of the trees used in the test were of a size to be graded first-class. Galled nursery trees have been seen that were short and very small in diameter, and the natural inference is that the presence of the disease accounted for the size of the tree. It is to be remembered, however, that trees of small caliper are not uncommon in the nursery even when no disease is present. Tree C-6 of the experiment is of this sort, and its behaviour when planted in the orchard is of particular interest. Two companion trees of C-6 were rejected for the experiment because their dwarfed condition obviously made them unfair to the test. One of the writers (R) planted these two trees in deep sandy loam on private land. Both trees have made excellent growth, and as they were top-worked to early-maturing varieties both have borne crops of fruit. One tree was removed in 1922 to make way for a building. There was no evidence of the original gall or of subsequent infection. The other tree is larger than some others that were set in the immediate vicinity two years earlier.

In connection with the outcome of this experiment the experience of a well-known horticulturist is of interest. The following extract is taken from an article by C. A. Green, for many years editor of *Green's Fruit Grower*. The article appeared in the issue of that journal for May, 1912. The orchard mentioned in the article is located twelve miles southwest of Rochester, New York, and is of special interest because it is in the main apple belt of western New York.

> Here is something that I want to call particular attention to. There is much said in these days about root gall on apple trees. At the time I planted this orchard but little was known about root gall. As I planted the trees with my own hands I noticed that occasionally a tree had a knotty excrescence on the roots which I now would recognize as root gall, and which is now regarded as infectious and fatal to the growth or welfare of the trees. Here is an interesting point which I desire to allude to: after growing for over thirty years and bearing fruit abundantly for over fifteen years, every tree in this orchard is healthy and one tree is as productive as another, and yet a number of these trees were infested with root gall. If the views of the experiment station are correct, the trees I planted thirty years ago, which were then affected with root gall, should long ago have perished or should have shown some signs of disease. That the trees are now all healthy and productive, teaches me that we have something yet to learn about root gall on apple trees.

THE EXPERIMENT WITH PEACHES

In the spring of 1911 a quantity of peach trees bearing evident lesions of crown-gall were procured from New York nurserymen. On April 6, 1911, photographs were made and the trees were set in the diseased garden as indicated in figure 3. Healthy trees were obtained in a separate shipment. The healthy trees were prepared for planting by one man, who used all antiseptic precautions. They were planted before the general lot of

infected trees, in order to avoid any contamination. All the trees were one year from the bud and were on peach stock. They were interplanted in the block of apples so that the whole block was on the quincunx plan with trees ten feet apart in the rows.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
A	**B**	**B**	**B**	**B**	B	B	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	
B		**B**		**B**		B		B		**B**		**B**		**B**		**B**		**B**	**B**
C	**B**	**B**	**B**	**B**	C	C	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**E**	**C**	**C**	**C**	**C**	
D		**C**		**C**		**C**		**C**		**C**		**E**		**C**		**C**		**C**	**C**
E	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	C	C	**C**	**C**	**C**	**C**	**C**	**C**	
F		**E**		**E**		**E**		**E**		**E**		E		E		**E**		**E**	**E**
G	**E**	**E**	**E**	**E**	**E**	**E**	**E**	**E**	**E**	**E**	E	E	**E**	**E**	**E**	**E**	**E**	**E**	
H		**F**		**F**		**F**		**F**		**F**		F		**F**		**F**		**F**	**B**

FIGURE 3. PLAN OF THE PEACH CROWN-GALL EXPERIMENT

Each character represents a tree and indicates the name of the variety according to the following key: *B*, Belle of Georgia; *C*, Carman; *E*, Elberta; *F*, Foster. Bold-face letters represent galled trees; light-face letters represent healthy trees

A great many of the trees failed to start growth when set, with the result that twenty-eight of the original ninety-eight affected trees were removed the first year. All of the affected trees of the variety Foster succumbed early. This may not be significant, because the original source and the treatment of these trees are not definitely known. From the high percentage of failures the first summer, it would appear that the crown-gall disease may be a factor in securing a good stand. This could be determined readily, but, unfortunately for the experiment, the crown-gall disease has been very scarce on peaches as well as on apples in New York nurseries since 1910, and doubtless for the same reason.

The winter of 1911-12 was severe, and winter sun-scald was abundant not only in the experimental block but throughout western New York. The loss from winter injury might be ascribed conveniently to a weakened condition of the trees due to the presence of crown-gall. The record shows, however, that 53 per cent of the healthy trees showed this injury, whereas only 20 per cent of the diseased trees were injured in this way. Furthermore, the winter of 1910-11 also was exceedingly severe for fruit trees of all kinds. Nurserymen generally were called on to replace many of the trees set in the spring of 1911. One firm alone replaced 20,000 peach trees out of a season's business of perhaps 300,000 trees. This may mean that two or three times the number replaced actually died, since, as a rule, not many persons demand replacement. For the most part the injury to the experimental trees occurred above the snow line, so that new growth started below the frost canker and above the point of union of scion with stock. Even when the renewal came from below the union, the tree was allowed to stand, since the development of the galls should be approximately the same.

In subsequent years many of the trees made excellent growth, as is shown in table 2, but each winter the temperature was low enough to kill fruit buds or else the buds were forced by a period of warm weather in late winter and then killed by frost.

The larvae of a borer (*Sanninoidea exitiosa*) gained entrance to a number of the trees in the late summer of 1913. As they were not noted until the following year, the number of trees showing effects of borer injury in 1914 is relatively large. The foliage of affected trees was slightly yellow (off color), was often somewhat rolled upward, and in some cases showed a marked tinge of red on the margins. Some of the borer channels extended far into the roots, and practically all of the trees removed on September 1, 1915, were injured primarily by borers. In some cases frost cankers were present, but these probably followed as a result of poor growth or immature wood consequent to borer injury.

Results of the peach experiment

From time to time the trees that were crowding, and especially those that were in poor condition, were removed. It was thus determined that borers were chiefly blamable for the poor appearance of certain trees, and that for the most part the galls had disappeared entirely. In many cases the position of the original gall could be determined only with difficulty or not at all. Usually the position could be determined by the occurrence on the stock of an area having the appearance of a healed wound (Plate II).

No crop of fruit was obtained during the course of the experiment, and so the only record is on the general appearance of the trees and the condition of the roots at digging time. The condition of the trees from year to year is shown in table 2. The same general reservations mentioned for the apples (page 7) apply in the case of the peaches, although the exact reason for the reservation is not the same in every instance.

The trees still living in the spring of 1918 were pulled. Unfortunately they were removed without warning and were not left in their regular order. An attempt was made to compare the leafless trees with previous growth records, but there was so much uncertainty about this that it was abandoned. Examinations of the pulled trees bore out the condition indicated in the table, namely, that most of the galls had disappeared.

The most interesting outcome of the experiment is that no peach trees died from the effects of crown-gall, and, so far as can be determined, the disease did not affect the growth of the trees to any appreciable extent. For the most part, galls either disappeared or were relatively small and insignificant. In a few cases the whole stock of the tree seems to have been enlarged, but even in such cases there was no indication, from the general appearance and growth of the tree, that any injury had been done.

TABLE 2. ANNUAL RECORD OF GROWTH AND DEVELOPMENT OF HEALTHY PEACH TREES AND OF TREES AFFECTED WITH CROWN-GALL

(The trees were set in the spring of 1911 and records were made from 1912 to 1917 inclusive. The final record was made on May 20, 1918. In examining the trees for general condition and appearance, four classes were recognized: *e*, excellent; *g*, good; *f*, fair; *p*, poor. Healthy trees are designated by an asterisk)

Variety	Location	1911 Oct. 3	1912 Oct. 12	1913 Sept. 6	1914 Sept. 14	1915 Sept. 14	1916 Sept. 11	1917 Sept. 25
Belle of Georgia	A- 1	.....	.....[1]	.....	.....	.....	.....	....
Belle of Georgia	A- 2	.....	f	f	g	g	g	f
Belle of Georgia	A- 3	Dead[2]	.....	.....	.....	.....	.....	.....
Belle of Georgia	A- 4	.....	g	g	f	p[4]	.....	.....
Belle of Georgia	*A- 5	.....	e	g	g	g	e	e
Belle of Georgia	*A- 6	.....	e	g	g	g	e	e
Belle of Georgia	A- 7	.....	e[3]	g	g	g	e	e
Belle of Georgia	A- 8	.....	e	g	g	g	e	e
Belle of Georgia	A- 9	.....	e	g	g	g	e	e
Belle of Georgia	A-10	.....	e[3]	g	g	g	e	e
Belle of Georgia	A-11	.....	f	g	g	g	g[5]	.....
Belle of Georgia	A-12	Dead[2]	.....	.....	.....	.....	.....	.....
Belle of Georgia	A-13	.....	g	g	g	g	e	e
Belle of Georgia	A-14	.....	g	g	g	g	e	e
Belle of Georgia	A-15	.....	g	g	g	g	g	g
Belle of Georgia	A-16	.....	g[3]	g	g	g	f[6]	.....
Belle of Georgia	A-17	Dead[2]	.....	.....	.....	.....	.....	.....
Belle of Georgia	A-18	.....	e[3]	f	f	f[7]	.....	.....
Belle of Georgia	B- 1	.....	e[3]	e	g	g	g[8]	.....
Belle of Georgia	B- 2	.....	e	e	e	e	e	e
Belle of Georgia	*B- 3	.....	e	g	g	e	e	g
Belle of Georgia	*B- 4	.....	e	g	e	e	e[9]	.....
Belle of Georgia	B- 5	.....	e[3]	g	e	e	e	e
Belle of Georgia	B- 6	Dead[2]	.....	.....	.....	.....	.....	.....
Belle of Georgia	B- 7	.....	g	g	e	g	g	e
Belle of Georgia	B- 8	Dead[1]	.....	.....	.....	.....	.....	.....
Belle of Georgia	B- 9	.....	e	g	e	g	e	e
Belle of Georgia	B-10	Dead[2]	.....	.....	.....	.....	.....	.....
Belle of Georgia	H-10	.....	e	e	e	f	g[10]	.....
Belle of Georgia	C- 1	.....	....[1]	.....	.....	.....	.....	.....
Belle of Georgia	C- 2	.....	e	g	g	g	g	f
Belle of Georgia	C- 3	.....	e	g	e	e	g	g
Belle of Georgia	C- 4	.....	e	g	g	e	e	e
Carman	*C- 5	.....	e	g	g	e	e	g
Carman	C- 7	Dead[2]	.....	.....	.....	.....	.....	.....
Carman	C- 8	.....	p[3]	g	g	f[11]	.....	.....

[1] Did not survive the winter.
[2] Failed to grow, or a few weak shoots pushed and then died.
[3] Girdled by frost; a new shoot developed from below the snow line.
[4] Removed September 1, 1915; winter injury; gall inactive, small.
[5] Removed October 11, 1916; crowding; good roots; crown considerably enlarged; several small galls.
[6] Removed October 11, 1916; roots fair; one small gall in crown; severe borer injury.
[7] Removed September 1, 1915; winter injury; gall disappeared.
[8] Removed October 11, 1916; excellent roots; large galls on crown; one gall 2 centimeters in diameter on one root.
[9] Removed September 11, 1916; excellent roots; no galls.
[10] Removed September 11, 1916; root system fair; crown somewhat enlarged but gall practically disappeared.
[11] Removed September 1, 1915; severe borer injury; gall disappeared.

TABLE 2 (*continued*)

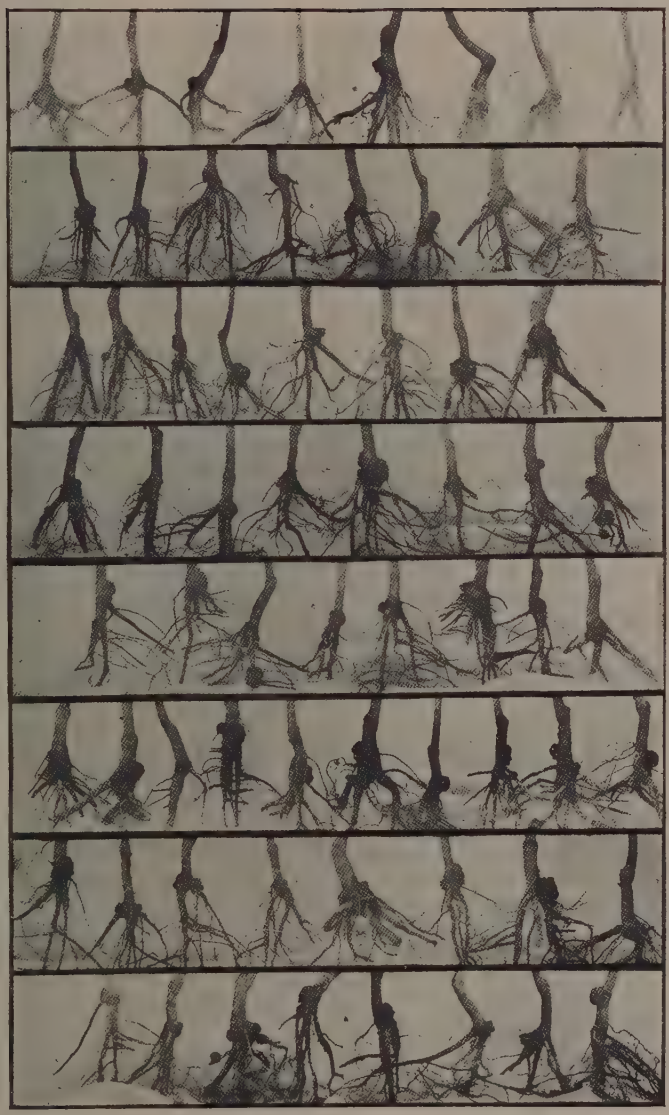
Variety	Location	1911 Oct. 3	1912 Oct. 12	1913 Sept. 6	1914 Sept. 14	1915 Sept. 14	1916 Sept. 11	1917 Sept. 25
Carman	C- 9	Dead[2]	.....	.....	.....	.....	.....	.....
Carman	C-10	.....	f	g	f	g	g	g
Carman	C-11	Dead[2]	.....	.....	.....	.....	.....	.....
Carman	C-12	Dead[2]	.....	.....	.....	.....	.....	.....
Carman	C-13	.....	g	g	f	e	e	e
Carman	C-15	.....	g	g	f	f[12]	.....	.....
Carman	C-16	.....	e	g	g	g[13]	.....	.....
Carman	C-17	.....	e	e	e	e	e	e
Carman	C-18	.....	f	g	p[14]	p[15]	.....	.....
Carman	D- 1	.....	e	e	e	e	e[16]	.....
Carman	D- 2	.....	g	g	e	g	e	e
Carman	D- 3	Dead[2]	.....	.....	.....	.....	.....	.....
Carman	D- 4	Dead[2]	.....	.....	.....	.....	.....	.....
Carman	D- 5	.....	Dead[1]	.....	.....	.....	.....	.....
Carman	D- 7	.....	e	e	e	e	e	e
Carman	D- 8	.....	f	p[14]	p	p	.....[18]	.....
Carman	D- 9	.....	g	g	g	e	e	e
Carman	D-10	.....	p[19]	g	....[20]	.....	.....	.....
Carman	E- 1	.....	g	g	e	g	e	f
Carman	E- 2	.....	g	e	e	e	e	e
Carman	E- 3	.....	p[3]	g	e	e	e[21]	.....
Carman	E- 4	Dead[2]	.....	.....	.....	.....	.....	.....
Carman	E- 5	.....	e	e	e	e	g[22]	.....
Carman	E- 6	Dead[2]	.....	.....	.....	.....	.....	.....
Carman	E- 7	.....	e	e	e	e	e	e
Carman	E- 8	.....	e	g	p	p	p[23]	.....
Carman	E- 9	Dead[2]	.....	.....	.....	.....	.....	.....
Carman	E-10	.....	g[3]	e	e	e	e	g
Carman	*E-11	.....	g	e	g	e	e	e
Carman	*E-12	.....	e	e	e	e	e	e
Carman	E-13	.....	e	e	e	e	e	e
Carman	E-14	Dead[2]	.....	.....	.....	.....	.....	.....
Carman	E-15	Dead[2]	.....	.....	.....	.....	.....	.....
Carman	E-16	.....	g	f	p[14]	f[24]	.....	.....
Carman	E-17	.....	e	g	g	f[25]	.....	.....
Carman	E-18	.....	g	g	g	e	e	e
Elberta	*C- 6	.....	g	g	g	g	e	g

[12] Removed September 1, 1915; severe borer injury; gall disappeared; two galls on roots; hairy root above crown.
[13] Removed September 1, 1915; original gall disappeared; three galls (walnut) on one root.
[14] Borer injury.
[15] Removed September 1, 1915; original gall disappeared; one gall 1 centimeter in diameter on one root.
[16] Removed October 11, 1916; gall disappeared; large tree, large root system.
[17] Removed October 11, 1916; root crown in bad condition, evidently from gall but complicated by severe borer injury; three small galls on roots.
[18] Missing. no record; severe borer injury previously.
[19] Injured by plow.
[20] Dead July 1, 1914; no record.
[21] Removed October 11, 1916; borer injury; several small galls.
[22] Removed October 11, 1916; borer injury severe; galls disappeared.
[23] Roots fair; three small galls and indications of hairy root.
[24] Removed September 1, 1915; original gall disappeared; one gall 1 centimeter in diameter on one root.
[25] Removed September 1, 1915; borer injury; gall disappeared; indication of hairy root.

TABLE 2 (*concluded*)

Variety	Location	1911 Oct. 3	1912 Oct. 12	1913 Sept. 6	1914 Sept. 14	1915 Sept. 14	1916 Sept. 11	1917 Sept. 25
Elberta	C-14	.....	f[3]	g	g	g	g[26]	.....
Elberta	D- 6	.....	f[3]	g	f	g	g[17]	.....
Elberta	F- 1	.....	g	f	p[27]	.....	.....	.....
Elberta	F- 2	.....	g	g	e	e	e	e
Elberta	F- 3	Dead[2]	.....	.....	.....	.....	.....	.....
Elberta	F- 4	Dead[2]	.....	.....	.....	.....	.....	.....
Elberta	F- 5	.....	f	f	f	f[28]	.....	.....
Elberta	F- 6	.....	e[3]	g	e	e	e	e
Elberta	F- 7	Dead[2]	.....	.....	.....	.....	.....	.....
Elberta	F- 8	.....	e	g	e	f[29]	.....	.....
Elberta	F- 9	Dead[2]	.....	.....	.....	.....	.....	.....
Elberta	F-10	.....	g	g	g	f	f	f
Elberta	G- 1	.....	f	f	e	g	g	e
Elberta	G- 2	Dead[2]	.....	.....	.....	.....	.....	.....
Elberta	G- 3	Dead[2]	.....	.....	.....	.....	.....	.....
Elberta	G- 4	.....	e	e	g	e	e	e
Elberta	G- 5	.....	e	e	g	g	e	e
Elberta	G- 6	.....	e	e	g	e	e	e
Elberta	G- 7	.....	g	e	e	e	g	e
Elberta	G- 8	.....	e	e	g	e	g	f
Elberta	G- 9	Dead[2]	.....	.....	.....	.....	.....	.....
Elberta	G-10	.....	g[3]	g	f	g	f[30]	.....
Elberta	*G-11	.....	g[3]	f	p[14]	p[31]	.....	.....
Elberta	*G-12	.....	g[3]	g	g	f	p[32]	.....
Elberta	G-13	.....	e	g	g	g	e	e
Elberta	G-14	.....	g[3]	g	p[14]	f	p[33]	.....
Elberta	G-15	.....	g[3]	g	g	g	e	e
Elberta	G-16	.....	g	g	g	f	e	e
Elberta	G-17	.....	e	g	f	f	e	e
Elberta	G-18	.....	g	f	g	f	g[34]	.....
Elberta	H- 8	Dead[2]	.....	.....	.....	.....	.....	.....
Elberta	H- 9	Dead[2]	.....	.....	.....	.....	.....	.....
Foster	H- 1	Dead[2]	.....	.....	.....	.....	.....	.....
Foster	H- 2	Dead[2]	.....	.....	.....	.....	.....	.....
Foster	H- 3	Dead[2]	.....	.....	.....	.....	.....	.....
Foster	H- 4	Dead[2]	.....	.....	.....	.....	.....	.....
Foster	H- 5	Dead[2]	.....	.....	.....	.....	.....	.....
Foster	*H- 6	.....	e[3]	g	g	f	g[35]	.....
Foster	*H- 7	.....	e	e	e	e	e	e

[26] Removed October 11, 1916; no record.
[27] Removed July 1, 1914; poor location and practically dead.
[28] Removed September 1, 1915; frost injury; gall disappeared.
[29] Removed September 1, 1915; injured by frost and borers; old gall not apparent.
[30] Removed September 11, 1916; borer injury; roots good; large galls, 6 to 7 centimeters in diameter, on crown and roots.
[31] Removed September 1, 1915; injured by borers and frost; practically dead.
[32] Removed September 11, 1916; severe borer injury; no galls.
[33] Removed September 11, 1916; borer injury severe; roots small; three small galls.
[34] Removed September 11, 1916; borer injury severe; one small gall.
[35] Removed September 11, 1916; recovering from severe borer injury; no galls.


Row A
Trees 1 to 4, and 10, 7, 8, 9
Row A
Trees 11 to 18
Row B
Trees 1, 2, and 5 to 10
Row C
Trees 1 to 4 and 7 to 10
Row C
Trees 11 to 18
Row D
Trees 1 to 10
Row F
Trees 1 to 5 and 8 to 10
Row G
Trees 18 to 13, 10, and 9

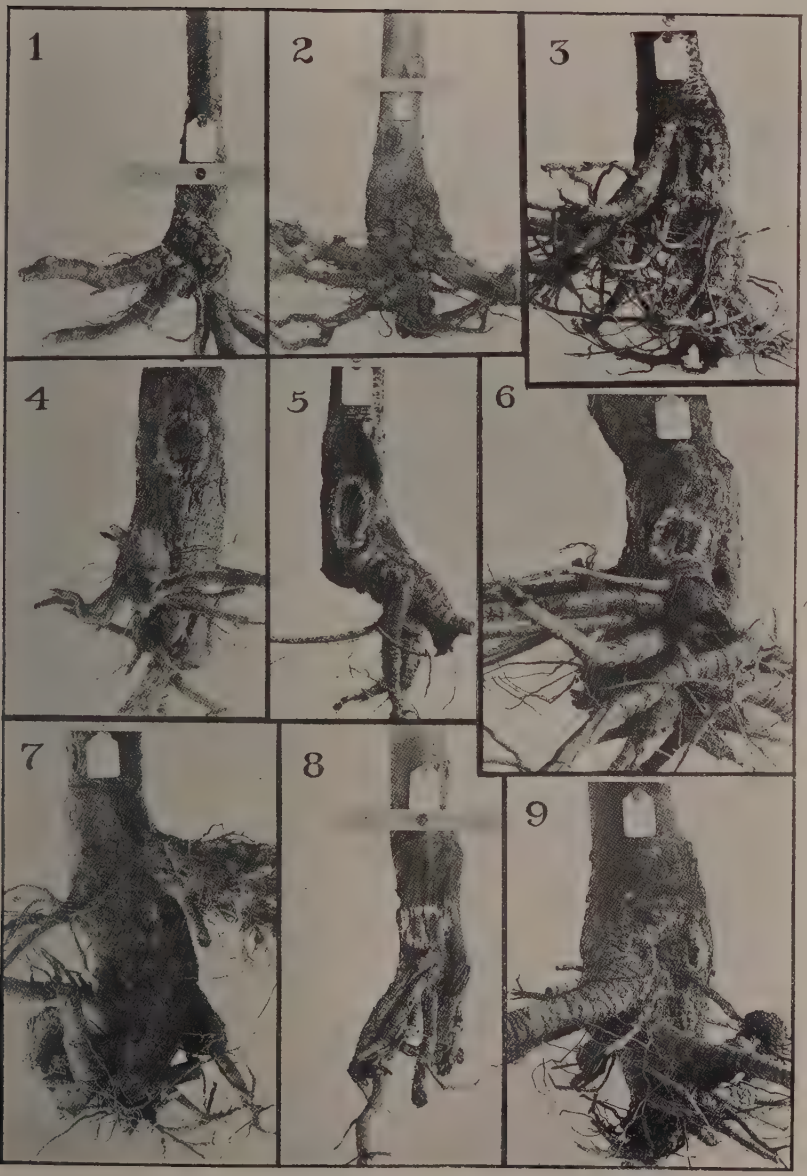
ROOTS OF THE PEACH TREES AFFECTED WITH CROWN-GALL WHICH WERE USED IN THE EXPERIMENT

The behavior of these trees in the field is shown in table 2. In each case read from left to right except in the row at the top, where tree 7 is tree 10 of the records and trees 8, 9, and 10 are 7, 8, and 9 of the records; and in the row at the bottom, where it is necessary to read from right to left. (Special acknowledgment is made to Mr. W. R. Fisher for his technical skill in obtaining suitable prints from most unsatisfactory negatives)

Plate II. Roots of peach trees from the crown-gall experiment

The condition of most of the roots at the time of setting is shown in Plate I, and a record of behavior previous to digging is given in table 2. In cases in which the original gall has disappeared, its location is outlined with chalk

1, Tree A-4. Removed September 1, 1915, because of winter injury. Gall apparently inactive
2, Tree A-11. Removed October 11, 1916, because of crowding. Crown considerably enlarged and several small galls, but tree apparently in good condition
3, Tree E-16. Removed September 1, 1915. A small gall at base of root issuing from near bottom of original gall
4, Tree A-18. Removed September 1, 1915, because of winter injury
5, Tree F-5. Removed September 1, 1915, because of frost injury. Gall disappeared, and no indication that it could have been a factor in decline of tree
6, Tree F-8. Removed September 1, 1915, because of injury by frost and borers
7, Tree G-11. Removed September 1, 1915, because of severe injury by frost and borers
8, Tree C-18. Removed September 1, 1915. Original gall disappeared
9, Tree C-16. Removed September 1, 1915. Original gall disappeared. Three new galls have appeared, two of which show clearly in the photograph



ROOTS OF PEACH TREES FROM THE CROWN-GALL EXPERIMENT

(See legend opposite)

GENERAL CONSIDERATIONS

Field experimentation is very unsatisfactory at best, because so many uncontrollable factors are involved which interfere with a correct interpretation of results. This condition has come to be rather generally understood, the recent analyses by Harris[10] of results from field plot tests, and by Anthony and Waring[11] for orchard tests, only serving to indicate how generally unreliable such data are. In addition to the usual vicissitudes encountered in plot testing, an additional set of variables is added when one attempts to compare plants in health and in disease. Means have been devised for overcoming heterogeneity in field plots, but not enough is known about plant responses under conditions of parasitism to make suitable corrections. This is particularly true in the case of the disease under consideration, because Smith has shown that the response to the stimulus of *Bacterium tumefaciens* depends a great deal on the particular tissue affected. This individual variation can be eliminated by increasing the number of plants under test. The number of plants involved in a wholly satisfactory experiment obviously should be considerably greater than was included in this experiment, and the arrangement should be different.

The general indication, however, is that under the conditions of this experiment, crown-gall is not a factor in the growth and development of either apple or peach trees.

The agitation among fruit growers in regard to crown-gall has practically disappeared, doubtless due to the inspection service which causes rejection of trees bearing galls. The writers, however, in 1914 received a consignment of 400 apple trees, of which 28 (7 per cent) bore definite lesions of crown-gall. All of these were planted on private land and their exact location was recorded. While it is likely that many growers treat galled trees just as the writers did, it is to be recalled that the amount of crown-gall on nursery stock has not been large, and the rejection of trees on account of crown-gall has not been a serious loss to nurserymen.

The rôle of crop rotation in reducing losses from crown-gall in the nursery is an interesting phase of the problem, on which, unfortunately, experimental data are too meager to warrant any conclusions.

[10] Harris, J. Arthur. Practical universality of field heterogeneity as a factor influencing plot yields. Journ. agr. res. 19:279–314. 1920.

[11] Anthony, R. D., and Waring, J. H. Methods of interpreting yield records in apple fertilization experiments. Pennsylvania Agr. Exp. Sta. Bul. 173:1–42. 1922.

BIOLOGICAL STUDIES OF BACTERIUM TUMEFACIENS

There are a number of biological problems connected with an investigation of crown-gall which are of considerable practical importance. One of the writers (S) was engaged in such work. He had undertaken an extensive series of tests designed to determine the longevity of the crown-gall organism in pure and in mixed culture, and under a variety of conditions. These were not completed when his connection with the institution was severed, and there was no opportunity to finish them before his untimely death intervened. The data accumulated are fragmentary, but their significance warrants publication at this time. They are given in the following paragraphs.

FIGURE 4. CROWN-GALL ON TOMATO PLANT

The cultures of *Bacterium tumefaciens* recovered in the studies on vitality were tested in this way in order to confirm identification

Persistence of B. tumefaciens in sterilized soil.— In order to determine the persistence of the crown-gall organism in sterilized soil, the following experiment was begun on November 10, 1914. Fifteen small pots were filled with loamy soil and sterilized. Five cubic centimeters of a bouillon culture of *B. tumefaciens*, four days old, was added to each pot. Twelve test tubes were partially filled, some with loam, others with quartz or clay, and to these one cubic centimeter of the culture was added. After four days all pots and tubes were tested to determine the presence in living condition of the organism (figure 4). All were then placed in a box out of doors. On January 2, 1915, the organism was recovered from clay, loam, and quartz. On April 14, 1915, a test of the loam soil in the test tubes showed the organism to be present in a living condition. Again on May 24, 1915, the organism was found to be alive in a pot of loam and in a tube of quartz. The next test was made on September 14, 1915. The organism was not recovered from either pots or tubes.

Persistence in unsterilized soil.—Experiments similar to the preceding were instituted with clay, loam, and quartz which had not been sterilized. As this was the most important series from the practical standpoint, it is unfortunate that data on the outcome cannot be found among Dr. Stewart's notes.

Moisture relation.—On February 24, 1914, tests were instituted to determine the vitality of *B. tumefaciens* in dry, moist, and saturated clay, loam, and quartz. Sterilized material in test tubes was used, and the tubes were constantly covered with a bell glass to prevent evaporation. On March 11, 1914, the organism was alive in all nine tubes. On April 10, 1914, the three tubes of dry material were tested and the organism was recovered. In a duplicate set of tubes which were not covered, the organism was dead on the latter date in the dry clay and the dry quartz. All the protected tubes were tested on November 18, 1914, and *B. tumefaciens* was found to be alive except in the tubes of dry clay and dry quartz. Sterilized wheat seeds were planted in some of the tubes of moist loam on April 17. The seeds grew and developed into plantlets. On May 29 a test was made of these tubes and *B. tumefaciens* was found to be alive.

Penetration of soil.—Tubes were filled with moistened quartz and with moist loam, and were sterilized. Five cubic centimeters of a culture of *B. tumefaciens* was poured into one end of each tube, and enough sterilized water was added so that water would drip from the other end. This was caught under aseptic conditions, and at the end of forty-eight hours was tested by plating. The organism was recovered from tubes 60 centimeters in length. No greater lengths were tested, as 60 centimeters represents the approximate penetration into the soil of roots of young trees. Similar tests with clay gave penetration in some instances and not in others. Apparently, if the clay became "puddled" from adding water too rapidly, the organism was filtered out.

While these tests are not extensive, they certainly indicate that, when free from competition, *B. tumefaciens* can live for several months in moist soil of various types, that it can withstand low temperatures and repeated change in temperature at or near the freezing point, and that it may move considerable distances with currents of soil water.

Memoir 67, *Observations on the Life History of Taphrocerus gracilis* (*Say*) (*Beetle, Family Buprestidae*), the sixth preceding number in this series of publications, was mailed on September 14, 1923.